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SIMULTANEOUS INFECTION IN A CHILD WITH TUBERCLE BACILLI OF THE HUMAN AND OF THE BOVINE TYPE*

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In 1913 I published a series of investigations concerning tuberculous infections in childhood.[1] This work constitutes in certain respects a continuation of similar investigations by Harbitz,[2] who has found with respect to tuberculosis in children up to fifteen years of age that 286 cases, or 59 percent, were free from tuberculous infection, while 198, or 41 percent, were infected. Among these 119, or 60 percent, died from tuberculosis; and 52, or 26.3 percent, had latent tuberculosis, and 27, or 13.2 percent, latent tubercle bacilli.

The most important results of my work so far may be summarized as follows: On injection into guinea-pigs of lymph glands obtained post mortem from 134 unselected cases, there resulted tuberculous infection in 52, or 38.8 percent, of the cases. In each individual case there were injected cervical and mesenteric lymph glands, in some cases also the bronchial glands. The percentage of tuberculous infection was lowest among children less than a year old and highest among the oldest.

According to age, the children investigated may be grouped as follows:

Age	Number of Cases Examined	Number of Cases Infected with Tuberculosis
Under one year	58	14—24.2 percent
One to three years	28	11—39.3 percent
Three to five years	14	7—50.0 percent
Five to fifteen years	34	20—58.8 percent
Total	134	52—38.0 percent

In twenty-eight of the cases infected with tuberculosis, the cause of death was tuberculosis. In fourteen cases, tuberculosis (latent form)

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[1] Norsk. Mag. f. Lægevidensk, 1913, 74, Supplement; reviewed in Deutsch. Med. Wchnschr., 1913, 39, p. 452.

[2] Jour. Infect. Dis., 1905, 2, p. 143; Norsk Mag. f. Lægevidensk., 1913, 74, p. 1.

was found as an incident at the post mortem; and in ten cases, tubercle bacilli were present in the lymph glands, in which morbid changes could not be demonstrated macroscopically or microscopically. In the majority of the cases, there seemed to be a general infection of the lymph glands, since there resulted a tuberculous infection from both groups of glands in 70.4 percent of the cases in which both groups were injected successfully into guinea-pigs.

Fifty of the cases of tuberculous children have been studied bacteriologically, tuberculous material being injected into guinea-pigs, and tubercle bacilli isolated in pure culture from the infected animals. The growth of the bacilli was then studied on glycerin broth, their virulence being tested by injection into rabbits, using at least two rabbits in each case; in several cases, calves were also injected.

In forty-six cases, tubercle bacilli of the human type were found; in three cases, of the bovine type; in one case, there was isolated a type of bacillus that was difficult to incorporate into either of the two main groups. This strain we had to regard at first as "atypical."

As will be observed from this brief résumé it is possible, in the great majority of the strains of tubercle bacilli that are isolated in cases of tuberculosis in children, to tell whether they should be classed under the human or the bovine type; it is seldom one encounters a strain that must be classed as atypical, in my work only one instance occurring.

As is well known there has existed a marked difference of opinion as to how these atypical strains are to be regarded. A number of investigators have declared that in the strains that reveal atypical characteristics, we have transition forms between the human and bovine types, in other words, the actual proof of the transformation in the human body of the bovine to the human type of tubercle bacillus. It has been stated, particularly by the English Tuberculosis Commission in their Final Report, that strains which appear as transitional forms cannot be regarded as such, because a closer study of the cultures may show that they are strains in which the bovine and human tubercle bacilli are mixed ("mixed virus").

Since the strain I have studied has been found to consist of both bovine and human bacilli, I wish to report the case with the results of the work connected therewith.

The culture was isolated from a mesenteric gland of a child, eight months old, who died of general tuberculosis. On isolation in the usual manner the bacilli from this case showed a rapid and luxuriant growth in glycerin broth and also proved to be acid-producing (Theobald Smith's reaction). In other words, the bacilli showed the cultural peculiarities characteristic of tubercle bacilli of the human type. Morphologically, the bacilli were long, slender, and beaded, such as one recognizes in the human type. The virulence of the bacillus is evident from the following experiment:

On May 12, 1910, a rabbit was injected subcutaneously over the abdomen with 0.01 gr. culture-substance from broth. On October 23, the rabbit was killed. It was greatly emaciated, the weight when injected being 2,750 gm.; when killed, 1,500 gm. At the site of injection was an abscess, as large as a hen's egg, filled with a large amount of thick, yellowish-white pus. In the inguinal and axillary lymph glands were a few tubercles. In the lungs were numerous tubercles of a gray and yellowish color, closely massed together, and reaching the size of a hempseed; there were also a few pea-sized nodules.

On April 22, a rabbit was injected intravenously with 0.001 gr. of the culture substance. After about five weeks the rabbit died. Both lungs showed a complete infiltration with yellowish, confluent tubercles; otherwise, no tuberculosis was found in the rabbit.

On Novemer 8, 1911, a calf was injected subcutaneously in the neck with 0.05 gr. of the culture substance of a growing broth culture. The calf, greatly emaciated, was killed three months afterward. At the site of injection was an abscess, the size of a child's head, filled with a yellowish pus in which tubercle bacilli were demonstrated in large numbers. On the neck and in the chest cavities, were numerous tuberculous glands, some as large as a walnut, and in both lungs were miliary tubercles and nodules the size of a pea.

As is evident from these results, we are dealing with a strain of tubercle bacillus which reveals morphologically and culturally the characteristics of the human type. On the other hand, the virulence test in animals indicates a virulence for animals not corresponding to the human type, the resulting tuberculosis in the rabbit and calf being too generalized. This strain therefore can not be unconditionally classified as either of human or bovine type, but must be designated as "atypical."

In order to study the strain more closely the following experiment was made:

Cultures were made from the abscess of the neck of the calf and also from the cervical lymph glands. Altogether twenty tubes of glycerin potato and ten tubes of glycerin serum were inoculated. After six weeks there appeared a scanty growth on three tubes of glycerin potato. Subcultures were made and finally, after several transfers, I succeeded in obtaining a surface growth on glycerin broth, the bacilli growing as a fine film on the surface, with only a few denser areas here and there. From a growing broth culture 0.001 gr. of culture substance was injected into a rabbit intravenously. The rabbit became greatly emaciated and died about three weeks after the injection, showing a great many densely packed tubercles in both lungs, together with a few tubercles scattered through one kidney.

It was therefore plain that the tubercle bacilli isolated from the tuberculous calf possessed the marked characteristics of tubercle bacilli of the bovine type, hence bovine tubercle bacilli must therefore have been present in the original culture.

Now in order to demonstrate whether bacilli of the human type were present in the original culture, I made a series of plate cultures, to obtain, if possible, isolated colonies of the human type. Making use of large, flat slices of glycerin potato, I succeeded on these in obtaining isolated colonies which could be used for my purpose. For the further study of the colonies, I selected the colonies that first grew, with the idea that from these I had the best prospect of obtaining the human type in pure culture, since this bacillus grows more rapidly and more easily on artificial media than the bovine bacillus.

From a plate culture with isolated colonies of bacilli, I selected six colonies and transplanted them on ordinary slanted glycerin potato media and after growth appeared I transferred the cultures to glycerin broth. All of these cultures proved to grow in a manner characteristic of the human type, producing a luxuriant growth on glycerin broth. In order to test the virulence, 0.01 gr. of the bacterial substance from each of the broth cultures was injected subcutaneously into rabbits, each of the cultures being tested with two rabbits. The results of these injections are given in Table 1.

It may be concluded from the table that there are three colonies (1, 2, and 5) in which the bacillus shows itself to be avirulent, or slightly virulent, toward rabbits, there being only unimportant lesions at the site of injection. Therefore these bacilli, which were isolated from the original culture, must be considered as of the human type. Of the remaining colonies, Colony 6 also showed itself to contain bacilli of slight virulence toward rabbits and yet toward one of the rabbits it showed virulence so severe that one cannot conclude with full assurance that this colony was pure, that is, that it consisted only of bacilli of the human type. As regards the other colonies, we may assume from the result of injections into rabbits, the injection of 0.01 gr. of culture substance having caused lesions of a greater or less degree in the internal organs, that the two types of bacilli were present together in these colonies.

TABLE 1

RESULTS OF INJECTION OF TUBERCULOUS BACTERIAL SUBSTANCE INTO RABBITS

Colony	Rabbits Injected Subcutaneously with 0.01 Gr. of Bacterial Substance	Date of Injection	Date Killed	Results of Autopsy
1	Rabbit 1. Weight, 2,100 gr....	5/24/13	9/ 3/13	Weight, 2,450 gr. At site of injection pea-sized nodule, partially caseated; no tuberculous changes of internal organs.
	Rabbit 2. Weight, 2,300 gr....	5/24/13	9/ 3/13	Weight, 2,220 gr. No lesion at site of injection; no changes in internal organs.
2	Rabbit 1. Weight, 2,210 gr....	5/24/13	9/ 5/13	Weight, 2,500 gr. A gray fibrous scar at site of inoculation; no tuberculous changes.
	Rabbit 2. Weight, 2,100 gr....	5/24/13	9/ 5/13	Weight, 1,950 gr. A pea-sized abscess at site of injection; no tuberculous changes of the internal organs.
3	Rabbit 1. Weight, 2,200 gr....	5/24/13	9/ 5/13	Weight, 1,700 gr. Numerous gray and yellow tubercles in both lungs; single tubercles in liver and spleen, none in kidneys; abscess of walnut size at site of injection; tuberculosis of regional lymph glands.
	Rabbit 2......................	5/24/13	9/ 5/13	Weight, 1,810 gr. Numerous gray, and a few yellow tubercles in the lungs, otherwise no tuberculosis of organs; small abscess at site of injection; tuberculosis of regional lymph glands.
4	Rabbit 1. Weight, 2,650 gr....	5/26/13	9/ 8/13	Weight, 2,320 gr. Single gray tubercles in lungs and spleen; walnut-sized callous abscess at site of inoculation; no tuberculosis of regional lymph glands.
	Rabbit 2. Weight, 2,480 gr....	5/26/13	9/ 8/13	Weight, 2,020 gr. In the right lung numerous, in the left few, gray and yellow tubercles; abscess scarcely of pea-size at site of injection; tubercles in regional lymph glands.
5	Rabbit 1. Weight, 2,300 gr....	5/26/13	9/12/13	Weight, 2,800 gr. Pea-sized caseous nodule at site of injection; no tuberculosis of organs.
	Rabbit 2. Weight, 2,180 gr....	5/26/13	12/ 9/13	Weight, 2,400 gr. No lesion at site of inoculation; no tuberculosis of internal organs.
6	Rabbit 1. Weight, 2,560 gr....	5/26/13	9/12/13	Weight, 2,800 gr. Millet-seed-sized nodule at site of inoculation; no tuberculosis of internal organs.
	Rabbit 2......................	5/26/13	9/12/13	Weight, 2,250 gr. Caseous nodule at site of injection; single tubercles in regional lymph glands; a few tubercles in both lungs.

CONCLUSION

The strain of tubercle bacilli isolated from the mesenteric gland of a tuberculous child which on first examination had to be classified as "atypical," upon closer study proved to be a "mixed virus," since both types of tubercle bacilli were isolated from the cultures. The individual from whom the cultures were isolated had been infected with tubercle bacilli both of the human and of the bovine type.